Ammonium analysis

Based on:

- *Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39:971-974.*
- *Steve Allison, Allison Lab Protocol: Nutrient Analysis, 1/2008*
- *BOWERC, . E., AND T. HOLM-HANSEN 1980. A salicylate-hypochlorite method for determining ammonia in seawater, Can. J. Fish. Aquat. Sci. 37: 794-798.*

The salicylate method is a variation of the Berthelot-Phenate method but does not require the use and disposal of toxic phenol. The salicylate method involves a three-step reaction sequence. The first reaction step involves the conversion of ammonia to monochloroamine by the addition of chlorine. The monochloroamine then reacts with salicylate to form 5-aminosalicylate. Finally, the 5-aminosalicylate is oxidized in the presence of sodium nitroferricyanide (a catalyst) to form a blue-green colored dye that absorbs light at 650nm

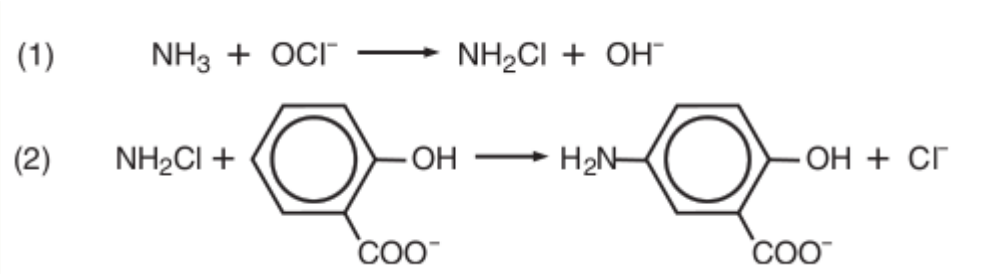

Fig 1: Ammonia compounds are initially combined with hypochlorite to form monochloramine (1),which then reacts with salicylate to form 5-aminosalicylate (2).

Note: This reaction is light sensitive. Perform in a dark environment (<85 lux) or add to every sample a calibration curve to counteract the degradation caused by light. For the same reason it is important to use a multichannel pipet to start every reaction at the same time.

BANANA GUARD

Protocols

Stock ammonium solution:
- 0.23585 g Ammonium sulphate/chloride
- 500 ml ultrapure water.

Sodium salicylate solution
- 6.8 g sodium salicylate
- 5.6 g Trisodium Citrate, Dihydrate
- 5 g Sodium Potassium tartrate, Tetrahydrate
- 0.025 g sodium nitroprusside (Disodium Pentacyanonitrosylferrate, Dihydrate)
- 100 ml ultrapure water

Sodium hydroxide solution
- 6 g sodium hydroxide
- 100 ml ultrapure water

Bleach solution (make fresh each day)
- 0.2 ml bleach (sodium hypochlorite)
- 9.8 ml sodium hydroxide solution

Standard curves: Dilute the 100 ppm stock solution to 10 ppm in a 1.5 ml centrifuge tube (150 µl
stock:1350 µl ultrapure water). Create the following standard curves in 1.5 ml centrifuge tubes.

High concentration (0-10 ppm)			Low concentration (0-1 ppm)		
Concentration	µl 10 ppm mix	µl water	Concentration	µl 10 ppm mix	µl water
0 ppm	0	1000	0 ppm	0	1000
0.5	50	950	0.05	50	950
1.0	100	900	0.10	100	900
2.0	200	800	0.20	200	800
5.0	500	500	0.50	500	500
10.0	1000	0	1.00	1000	0

Detection limit <0.05 ppm

For 96-well plate (~200µl):
For low concentrations (0-5 ppm):
Add the following to each well:
1. 80 µl sample
2. 60 µl bleach solution (add using multichannel pipet)
3. 60 µl salicylate solution (add using multichannel pipet)

For high concentrations (1-10 ppm):
1. 20 µl sample
2. 90 µl bleach solution (add using multichannel pipet)
3. 90 µl salicylate solution (add using multichannel pipet)

Pipet up and down to mix well, incubate 50 min and read plate at 650 nm.